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Tangier Disease (Familial High Density Lipoprotein Deficiency)*

Clinical and Genetic Features in Two Adults

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In 1961 Fredrickson and associates [*1,2*] described a new disorder of cholesterol and lipoprotein metabolism in two young siblings. The significant clinical features were hypocholesterolemia and enlarged tonsils of unusual appearance. One sibling also had hepatosplenomegaly and lymphadenopathy. Foam cells observed in the tonsils and lymph nodes contained large amounts of cholesterol esters. Both siblings were found to have nearly complete absence of plasma high-density or alpha$_1$ lipoprotein (HDL). In the absence of further knowledge of the basic defect, the disorder was named "Tangier disease," after Tangier Island, the Chesapeake Bay home of the family. More recently, two other cases in children of a family in Missouri unrelated to the Tangier Island patients have been discovered [*3,4*].

Studies of the kindreds of these two involved sibships revealed that many family members had HDL concentrations well below those of comparable control subjects [*4*]. The data appeared to be consistent with the hypothesis that control of plasma HDL concentrations resides in a single pair of autosomal genes. The phenotype resulting from a single abnormal mutant is characterized by low HDL concentrations; the homozygous individual exhibits the full blown syndrome of tissue storage of cholesterol esters and has no significant plasma HDL.

The present report describes the fifth and sixth cases of Tangier disease, which involve two brothers. These are the first cases observed among adults and provide new insight into the manifestations and course of this hereditary disease. Included also are the results of plasma lipid and lipoprotein determinations among members of this kindred.

REPORT OF CASES

CASE 1. A forty-two year old, white, oil-refinery worker who first registered at the Mayo Clinic in March 1962 was referred by his family physician for the evaluation of splenomegaly. He had consulted his physician in the fall of 1961 because of fatigue, at which time mild anemia and splenomegaly were found. The anemia was said to have diminished following iron therapy, but splenomegaly and mild fatigue persisted. The patient had been well otherwise and able to work regularly; no other symptoms were elicited except for intermittent mild diarrhea of several years' duration, manifested as two to four soft to loose, floating, occasionally foul-smelling stools daily. The history disclosed none but the usual childhood illnesses. He had undergone tonsillectomy at age fourteen in a physician's office, but recalled little about prior indications except recurrent sore throat and the judgment that his tonsils were quite enlarged. A cyst on the thyroglossal duct had been removed in 1945 while he was in naval service.

On physical examination, the patient appeared generally healthy and alert. Weight was 172 pounds and height 68 inches. Blood pressure was 135 mm. Hg systolic and 90 diastolic. No skin lesions were noted except for a few hyperpigmented areas over the anterior tibial regions. The corneas had a slightly cloudy appearance. Slit-lamp examination revealed the clarity and relucence of the corneas to be definitely diminished, with random soft densities involving the entire stromal thickness of each cornea. In the pharynx were several flat patches of yellow-orange lymphoid tissue. No lymphadenopathy was noted. The splenic tip was palpated 6 to 8 cm. below the left

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TABLE I
LABORATORY FINDINGS IN TANGIER DISEASE

Tests	Results		
	Normal	Case 1	Case 2
Hemoglobin (gm./100 ml. blood)	14–17	13.2	14.2
Erythrocytes (million cu. mm. blood)	4.2–5.5	4.73	4.65
Leukocytes (cu. mm. blood)	5,000–9,000	3,300	6,000
Sedimentation (mm. hr., Westergren)	0–20	10	...
Reticulocytes (per cent)	0.5–2.5	2.5, 10	...
Bromsulfalein retention (per cent)	<6	8	0
Prothrombin time (sec.)	17–19	21	20
Iron (μg./100 ml. serum)	50–175	120	126
Sugar (mg./100 ml. blood)	65–90	84	76
Bilirubin (mg./100 ml. serum)			
Direct	0	0	0
Indirect	<0.6	1.5	1.94
SGOT (μM/hour/ml.)	<1.43	1.61	1.34
Phosphatase			
Alkaline (King-Armstrong units/100 ml. serum)	<14	17	5.9
Acid			
Total (Bessey-Lowrey units)	0.13–0.63	0.45	0.34
Tartrate-inhibitable (King-Armstrong units/100 ml. serum)	<0.7	0.07	0.09
Carotene (μg./100 ml. serum)	>69	26.9	27
Calcium (mg./100 ml. serum)	8.9–10.1	10.0	9.6
Uric acid (mg./100 ml. serum)	3.8–7.1	9.1, 9.6*	9.0, 10.1
Protein (gm./100 ml. serum)			
Albumin	3.3–4.3	4.27	4.28
Alpha$_1$ globulin	0.3–0.4	0.48	0.36
Alpha$_2$ globulin	0.5–0.8	0.5	0.43
Beta globulin	0.6–1.1	0.71	0.93
Gamma globulin	0.8–1.6	0.94	1.21
Fecal nitrogen (gm./day†)	<2.5	1.6	0.75
Fecal fat			
gm. per day†	<7.5	9.66	4
Per cent of fecal solids†	>30	30	17

* By uricase method, 9.4 (courtesy of Dr. J. Seegmiller).
† 72-hour collection while on 100 gm. fat diet.

costal margin, and the liver edge was noted 2 to 3 cm. below the right costal margin.

General laboratory findings are given in Table I. (Plasma lipids and lipoproteins are described in a later section.) A smear of the peripheral blood showed anisocytosis, polychromasia, mild leukopenia and thrombocytopenia. Biopsy revealed that the sternal marrow was active, with many foci of foam cells surrounded by excess of eosinophils. The chest roentgenogram did not show any abnormality. The cholecystogram revealed a normally functioning gallbladder. Roentgenograms of the long bones and of the stomach, small intestines and colon did not show any defects.

Proctoscopy (by Dr. R. J. Spencer) revealed the rectal mucosa to be diffusely pale yellow and studded with tiny flat, discrete, orange-brown spots throughout that portion of the bowel visualized. Biopsy of the rectal mucosa showed large histiocytes filled with foamy cytoplasm in the mucosa and submucosa. Peroral biopsy of the jejunal mucosa, using the Crosby-Kugler capsule, did not disclose any abnormalities. A diligent search was made for foam cells or accumulated intracellular lipid in the biopsy specimen, but none was seen, not even with special stains. A liver biopsy specimen showed one small intralobular cluster of foam cells. Special lipid staining of the biopsy specimens of the liver and rectal mucosa (by Dr. A. H. Baggenstoss) showed that the foam cells and some small granules in the hepatic parenchymal cells reacted positively with the oil red O stain and gave a faint positive reaction to the periodic acid-Schiff stain. The Schultz reaction for cholesterol showed small foci of blue-green coloration in the

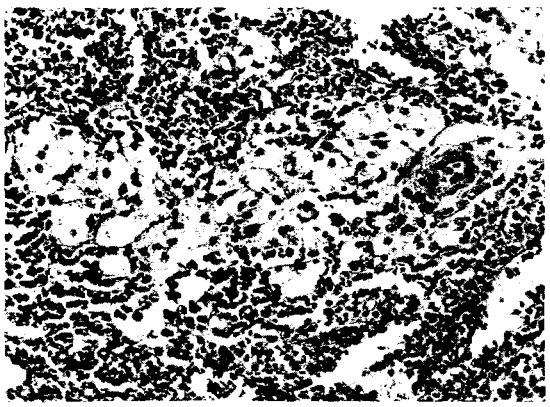

FIG. 1. Case 1. Spleen showing lipid-filled reticuloendothelial cells of pulp. Hematoxylin and eosin stain, original magnification × 250.

parenchymal cells and brilliant blue-green staining of the foam cells in the rectal tissue.

No abnormalities were noted (by Dr. J. A. Gregg) in the relative proportions of the unconjugated bile acids and their taurine and glycine conjugates in a specimen of bile obtained by duodenal intubation [5]; the total bile acid concentration was within the lower range of normal. The free amino acids in a 24-hour urine sample were normal, and the alpha-amino nitrogen content was 210 mg. (as determined by Dr. J. D. Jones). The serum contained normal amounts of total and alpha$_1$ and alpha$_2$ globulin glycoprotein [6] (as established by Dr. W. F. McGuckin).

Since then, the patient has been re-evaluated at the Mayo Clinic or National Institutes of Health every three to six months. Plasma cholesterol values have remained low. The spleen has increased in size, and on each visit the patient has complained of increased upper abdominal fullness and discomfort. Thrombocytopenia and leukopenia have become more marked, along with reticulocytosis and other evidence of increased regeneration of erythrocytes, although hemoglobin values have not decreased significantly.

In September 1963 the patient complained of disabling physical discomfort produced by the spleen, the inferior margin of which was then below the level of the umbilicus. The liver had not increased in size since the initial examination. Splenectomy was performed on September 7. At operation, the spleen was found to be greatly enlarged and much firmer than normal. It weighed 1,160 gm. and was yellowish red. The liver was only slightly enlarged and appeared normal grossly. There were many enlarged lymph nodes along the entire root of the mesentery. The other findings at intra-abdominal exploration were within normal limits. Microscopic sections of the spleen showed the reticuloendothelial cells of the pulp to be filled with intracytoplasmic lipid droplets. There were also scattered clusters of cholesterol crystals in the pulp. (Fig. 1.) Biopsy of a lymph node showed marked fatty change. The results from analysis of spleen lipids are shown in Tables II and III. The patient's convalescence was uneventful, and he resumed a normally active life.

In February 1964 he noted enlargement of the right breast. Other findings on physical examination at this time were within normal limits. Excisional biopsy of the right breast disclosed benign hyperplasia of the ductile tissue and stroma. Staining for fat did not reveal any foam cells in the resected tissue. Laboratory studies on two occasions showed the alkaline phosphatase to be 28 and 34 King-Armstrong units. The bromsulfalein test showed 20 per cent dye retention. The plasma total cholesterol was 125 mg. per

TABLE II
SPLEEN LIPIDS (MG./GM. WET TISSUE)

Data	Case 1	Controls*	
		A	B
Total lipid	50.7	20.6	22.2
Phospholipid	15.4	10.4	14.7
Phosphatidyl choline	4.6	2.1	4.1
Sphingomyelin	2.3	2.0	1.9
Cholesterol			
Free	4.8	4.0	3.3
Esterified	7.8	0.7	0.2
Cholesterol-ester fatty acids (estimated)	5.5	0.5	0.1
Triglyceride	2.0	0.4	0.4
Lipid hexose	0.2	0.2	

* Control tissues were obtained from (A) a child and from (B) an adult who had died following cardiac surgery. Both tissues showed histologic evidence of chronic passive congestion.

TABLE III
FATTY ACIDS IN CHOLESTEROL ESTERS FROM SPLEENS

Fatty Acid*	Case 1 (%)	Control B† (%)
12:0	1.5	...
14:0	5.7	0.8
15:0	4.9	0.3
16:0	21.8	31.1
16:1	13.7	4.2
17:0	0.6	...
18:0	4.1	13.0
18:1	40.0	21.9
18:2	5.1	19.6
20:4	3.6	9.0
24:1	1.9	...

* Number carbons in chain: number double bonds.
† Source as in Table II.

100 ml., and on paper electrophoresis an increase in low-density lipoprotein (LDL) concentration was noted.

CASE 2. A farmer and distillery worker, the forty-eight year old brother of the first patient, was seen at the Mayo Clinic in March 1963 after a preliminary survey of the family led to the suspicion that he also might have Tangier disease. His medical history included tonsillectomy in 1950 because of recurrent sore throat and "enlarged tonsils." (The surgical transcripts were obtained from his local hospital, but there was no comment about the unusual features of the tonsils and no histologic studies were reported.) Typical angina pectoris of moderate severity had been noted daily for the past five years. A prolonged episode of chest pain had led to the patient's hospitalization near his home in 1961, but "no permanent damage" had been noted. Dull, nonspecific, right abdominal discomfort of several years' duration was the patient's only other current complaint. No history of diarrhea or steatorrhea could be elicited.

On physical examination, the weight was 208 pounds and height 67 inches. The blood pressure was 142/100 mm. Hg. The only skin lesions were two 3 mm. yellow-brown spots on the lower calf and tibial areas. There was no corneal clouding on gross examination, but slit-lamp examination showed a hazily flocculent infiltration throughout the corneal stroma. This was described by an ophthalmologist observer (Dr. Ludwig Von Sallmann) as "decreased transparency of the cornea, more marked centrally than peripherally, and more prominent in the posterior than the anterior stroma. This slight diffused opacity can be resolved into fine equidistant dots." Funduscopic examination revealed minimal sclerosis of the retinal arterioles in both eyes and a fluffy retinitic spot in the inferior temporal region of the left. Cystic limbal lesions thought to be pingueculae were present bilaterally. In the pharynx a few follicles remained, surrounded by a pale yellow-orange ring in several instances. The liver edge was palpable at the right costal margin; the spleen was not palpable. The other physical findings were not remarkable.

General laboratory findings are listed in Table 1. The standard thoracic roentgenogram, roentgenograms of the gallbladder, colon and lumbar vertebrae and the excretory urograms did not reveal any abnormality. An electrocardiogram showed a nonspecific repolarization abnormality in the mid-precordial leads. Marrow aspirated from the iliac crest contained a few macrophages ("foam cells"), their cytoplasm loaded with doubly refractile droplets. Many other scattered droplets appeared to be outside any cytoplasmic membrane. The remaining marrow elements were normal. Proctoscopic examination revealed a smooth yellowish mucosa with tiny, flat, dark red-brown 1 mm. spots scattered throughout. A rectal biopsy specimen showed mucosal and submucosal collections of foam cells. Biopsy of tonsil and adenoid remnants revealed clusters of pale-staining foamy macrophages. A jejunal biopsy specimen did not show any histologic abnormalities, but a needle biopsy specimen of the liver revealed one small intralobular focus of foam cells, as in the previous case. The foam cells from pharyngeal, rectal and liver biopsy specimens exhibited histochemical characteristics identical with those in Case 1. (Fig. 2.)

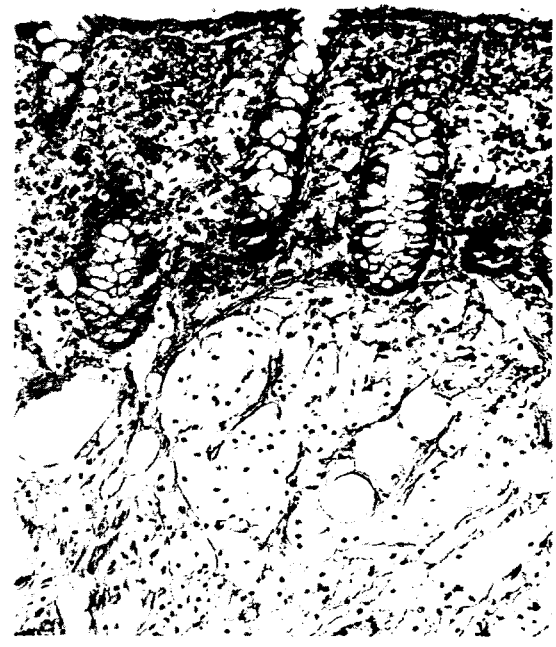

FIG. 2. Case 2. Rectal biopsy specimen showing large lipid-laden cells in lamina propria and beneath muscularis mucosa. Hematoxylin and eosin stain, original magnification × 150.

The patient's symptomatic coronary heart disease was discussed with him, and he was also advised to reduce his weight. On July 22, 1963, he died suddenly while driving his farm truck. Necropsy was not performed.

METHODS

Measurements of cholesterol [7], phospholipid [8] and triglycerides [9] were performed on chloroform-methanol extracts [10] of tissue, plasma or lipoprotein fractions isolated by preparative ultracentrifugation [11]. Tissue was homogenized in chloroform: methanol (2:1), using two 10 to 20 ml. portions of solvent per gram of wet tissue. After the combined extracts were filtered and the chloroform phase washed according to the method of Folch and associates [12], they were kept at 4°C. until analyzed. Total lipid was determined gravimetrically and lipid hexose was measured by the anthrone method [13] following

TABLE IV
PLASMA LIPIDS (MG./100 ML.)

Source	Cholesterol		Phospholipids	Triglycerides
	Total	Free		
Case 1	38 (30–45)	14	86 (68–104)	142 (136–152)
Case 2	69 (50–88)	21	114 (104–124)	213 (195–238)
Controls	250 (176–324)	...	280 (205–355)	70 (20–120)

NOTE: Values for phospholipids and triglycerides of patients represent two to five postabsorptive samples obtained on different days. Control cholesterol and phospholipid values represent mean (and 90 per cent confidence limits) obtained from forty-three normal men forty to forty-nine years old, averaging 44.5 years of age. Control triglyceride values were derived from major peak of distribution of values obtained from 390 fasting and apparently healthy subjects [*23*].

TABLE V
ULTRACENTRIFUGAL ANALYSIS OF LIPOPROTEINS
(MG./100 ML. PLASMA)

Lipoprotein Fraction	Case 1		Case 2		Controls*	
	C†	PL†	C	PL	C	PL
Plasma	30	68	50	104	171	215
<1.019	19	44	33	70	26	31
1.021–1.063	11	15	13	23	97	69
1.063–1.21	1	2	<1	3	43	111
>1.21	0	7	<1	8	0	21

* Mean of samples from ten men, aged twenty-one to twenty-eight years. From Havel et al.[*11*].
† C = cholesterol. PL = phospholipid.

hydrolysis of the sample at 100°C. for 15 hours in 6 N hydrochloride. Phospholipids were separated and quantified by the method of Skidmore and Entenman [*14*] using two-dimensional thin layer chromatography. Cholesterol esters were isolated by chromatography on alumina. Methyl esters of fatty acids were chromatographed on 15 per cent ethylene glycol succinate at 195°C. The ionization detector was calibrated for response with NIH Metabolism Study Section standards. Paper electrophoresis for lipoproteins was performed by the technics of Swahn [*15*] and of Lees and Hatch [*16*].

RESULTS

Plasma Lipids and Lipoproteins. In the two cases, the plasma lipids (Table IV) followed the abnormal pattern which is characteristic of Tangier disease, although not necessarily unique. This is the association of low levels of cholesterol and phospholipids with normal or elevated concentrations of glycerides. On the basis of the known composition of the major plasma lipoprotein systems, it may be predicted from this lipid pattern that there will be a deficiency in HDL or in that portion of LDL of density between 1.019 and 1.063, with an associated finding of normal or elevated concentrations of triglyceride-rich LDL of density less than 1.019.

In these cases all three features proved to be present. On paper electrophoresis, no alpha$_1$ lipoprotein could be seen. On preparative ultracentrifugation (Table V), there was no significant HDL in the appropriate density band, 1.063 to 1.21. Also, there was a definite decrease in LDL of density 1.019 to 1.063 (which corresponds to beta lipoproteins on paper electrophoresis or Sf 0 to 12 in the analytical ultracentrifuge) and probably a significant increase in LDL of density less than 1.019 in Case 2.

Immunoelectrophoresis on agar, using either rabbit antibodies to human HDL or horse anti-human serum, revealed no precipitation line attributable to HDL. The results of agar diffusion studies are shown in Figure 3A. The rabbit anti-HDL serum used in these experiments has

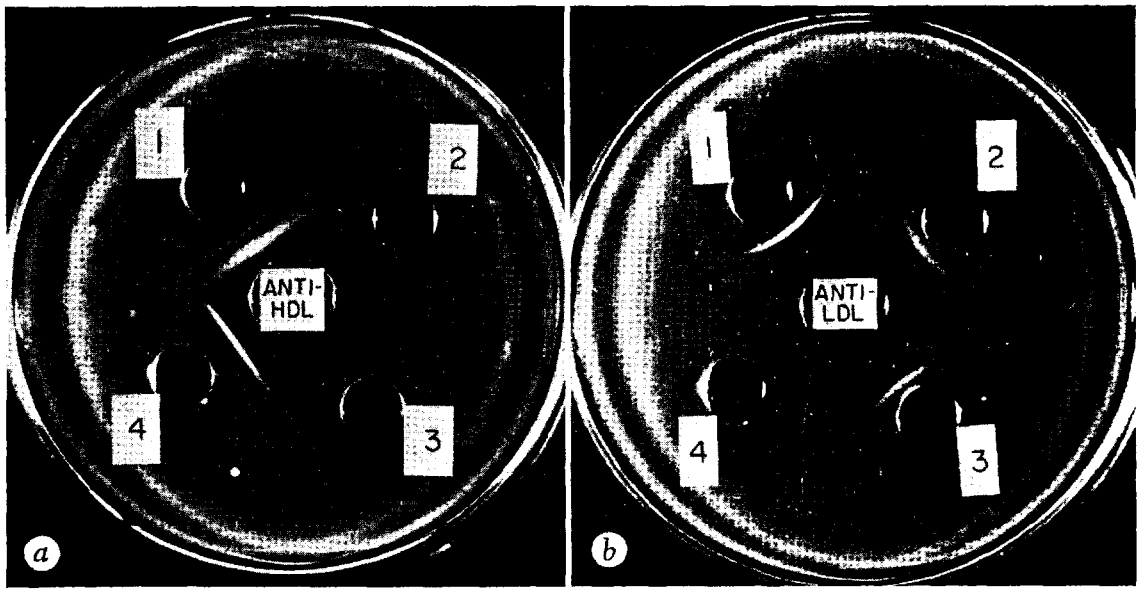

FIG. 3. A, precipitation lines formed by Ouchterlony agar diffusion technic with serum from normal subject (1), Case 1 (2), Case 2 (3) and patient with acanthocytosis and abetalipoproteinemia (4) against rabbit antibodies prepared against human lipoproteins of density 1.063 to 1.21 ("anti-HDL"). Both precipitation lines at (1) are attributed to HDL (see text). B, arrangement same as in A, except rabbit antibodies were prepared against human lipoprotein of density 1.063 ("anti-LDL"). Here both patients with Tangier disease demonstrated LDL, which was further shown by immunoelectrophoresis. These plates were prepared by Dr. Robert I. Levy.

been shown to detect two different HDL antigens and to contain no antibodies to either albumin or LDL [*17*]. A very faint precipitation line was formed by serums from both patients, suggesting the presence of a very small amount of HDL. (Fig. 3A.) This is very similar to results obtained in the four other known cases of Tangier disease, and precise identification of the faintly detectable antigen is under way. In contrast to serum from a patient with abetalipoproteinemia (kindly supplied by Dr. Leonard Laster), serums from both of the present patients had easily detectable LDL, antigenically similar to that in normal subjects although somewhat decreased in amount. (Fig. 3B.)

The abnormal concentrations in the LDL fractions were further associated with lower than normal cholesterol:phospholipid ratios in these lipoproteins. Both of these abnormalities have also been observed in lesser degree in the four children with Tangier disease [*18*].

Tissue Lipids. The significant splenic abnormality in Case 1 (Table II) was the presence of cholesterol esters in amounts which far exceeded that in the two control tissues analyzed concurrently. The fatty acid pattern of the cholesterol esters in the spleen in this case (Table III) was similar to that seen earlier in tonsils and lymph nodes from the children with Tangier disease [*2*]. Oleic acid predominated and the relative amounts of linoleic and palmitic acids were less than those seen in the control spleen. (Table III.)

A considerable amount—about 30 per cent—of the total lipid of the spleen in Case 1, as estimated gravimetrically, could not be accounted for in the summation of the cholesterol, phospholipid and glyceride fractions. (Table II.) Such measurements were made a total of nine times on three different extracts of the abnormal spleen. Since 15 to 22 per cent of lipid was unaccounted for in the controls also, it is likely that the gravimetric measurements of total lipid were uniformly and inexplicably too high; but the possibility of an abnormal and undetected lipid in the tissue in Case 1 has not been excluded completely.

The lipid hexose content was normal, however; and the total fatty acid content, determined by quantitative gas chromatography after saponification and methylation, was equivalent to that predicted from the quantities of esterified lipids specifically measured and presented in Table II. The gas chromatographic pattern of the total fatty acids in the spleen

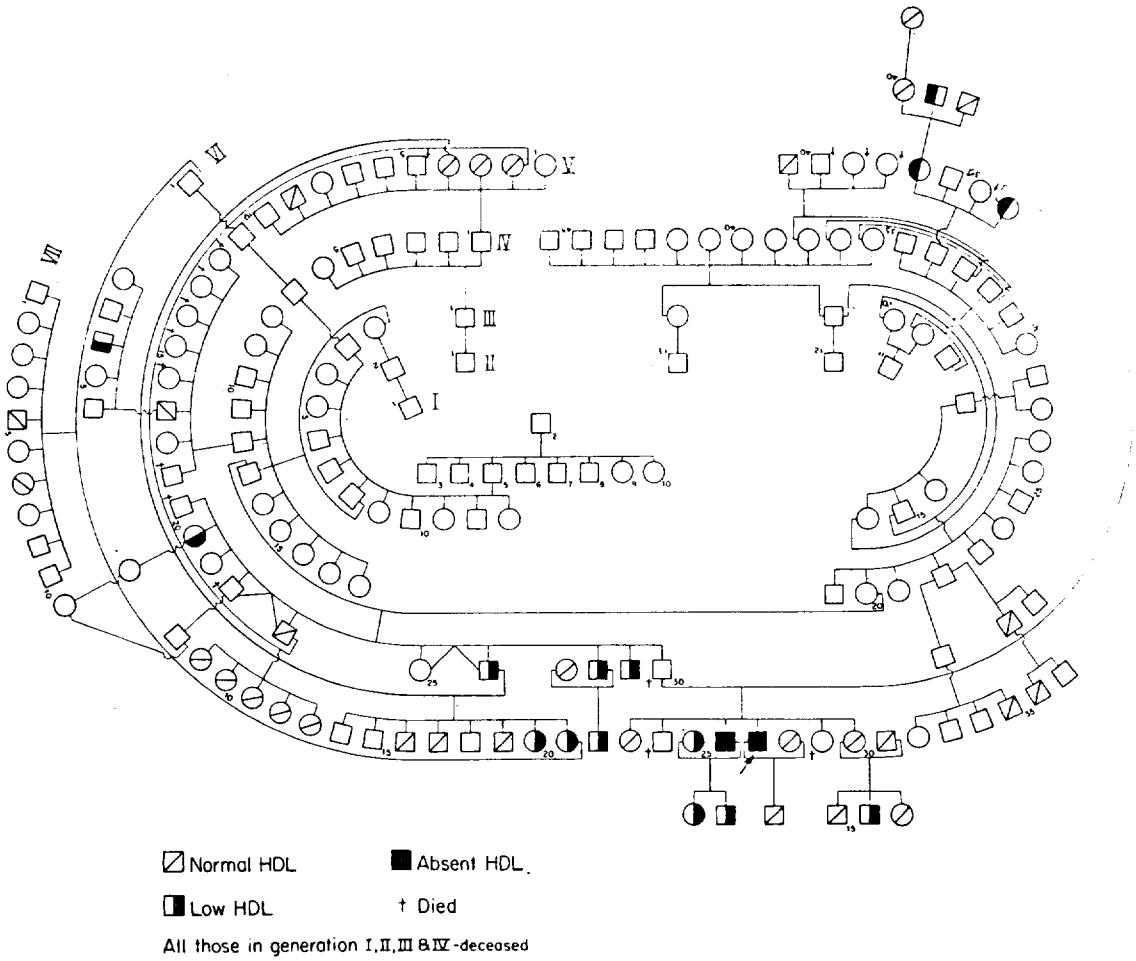

FIG. 4. Pedigree of Kentucky propositi, showing distribution of HDL concentrations among forty-eight relatives. See Table VI for details of HDL values.

differed significantly from that of control tissue (spleen B, Table III) only in an increased percentage of oleic acid, attributable to the increased amount of this acid esterified with cholesterol. Within the limits of detection of the methods employed, the abnormality in lipids from the spleen in Case 1 was qualitatively the same as that previously found in other tissues from children with Tangier disease [*2,18*].

STUDY OF THE KINDRED

The patients described herein are two of six children born to parents of seemingly pure English ancestry, both of whose families have lived in Kentucky for several generations. Two female siblings are living. (VI–23, VI–30, Fig. 4 and Table VI.) A male and a female sibling each died at about two years of age from "throat trouble" said possibly to have caused them "to choke to death." The two surviving sisters of the patients appear to be in good health. The tonsils of one have been removed but no records concerning them are available. The tonsils of the other appear normal. Their mother (V–34, Fig. 4 and Table VI) is seventy-nine years of age and in good health. The patients' father (V–30, Fig. 4 and 5) died of a myocardial infarction at the age of seventy-five.

Most of the kindred reside in a small area of western Kentucky, into which their forebears migrated in the late eighteenth and early nineteenth centuries. Nearly all their contemporary associates likewise were of Anglo-Saxon ancestry and crossed the Appalachians from the eastern seaboard states. Antecedents of the present kindred have been traced to Pennsylvania,

TABLE VI
DISTRIBUTION OF LIPID FRACTIONS AMONG KENTUCKY KINDRED

No.	Age (yr.) and Sex	Plasma			Density >1.063		HDL Phenotypic Classification†
		TC*	PL*	TG*	TC	PL	
v–2	79, F	280	297	...	66	144	N
v–3	77, F	299	347	...	50	128	N
v–4	75, F	344	376	145	72	170	N
v–9	64, M	294	331	...	64	158	N
v–17	71, M	174	250	176	33	97	N
v–21	85, F	167	218	148	22	74	L
v–24	79, M	261	273	209	34	94	N
v–26	75, M	181	242	215	24	82	L
v–27	56, F	221	313	...	49	144	N
v–28	69, M	...	...	...	15	72	L
v–29	68, M	237	279	155	19	75	L
v–32	68, M	373	353	...	53	122	N
v–34	79, F	160	190	131	30	87	L
v–37	87, F	118	166	116	20	67	L
v–41	73, M	205	248	...	52	136	N
vi–4	36, M	229	301	290	30	91	L
vi–9	56, F	213	305	178	53	138	N
vi–10	57, F	418	389	...	79	184	N
vi–11	55, F	245	315	...	59	161	N
vi–12	48, F	250	299	...	81	171	N
vi–13	42, F	284	304	...	58	131	N
vi–16	49, M	232	281	84	117	160	N
vi–17	43, M	174	274	174	34	115	N
vi–19	35, M	234	316	213	37	107	N
vi–20	46, F	215	266	104	29	93	L
vi–21	45, F	132	176	...	33	93	L
vi–22	36, M	157	222	163	29	87	L
vi–23	58, F	278	347	...	44	121	N
vi–25	45, F	241	298	...	30	101	L
vi–26	48, M	88	115	216	1	14	A
vi–27	44, M	36	104	138	1	8	A
vi–28	43, F	199	220	49	52	111	N
vi–30	36, F	115	159	54	35	89	N
vi–31	34, M	228	233	...	35	85	N
vi–35	36, M	252	317	...	44	134	N
vi–36	37, M	306	258	...	53	104	N
vi–38	60, M	226	243	...	39	107	N
vi–39	69, M	236	287	216	31	100	L
vi–40	65, F	241	331	...	58	148	N
vii–5	19, M	136	187	...	36	102	N
vii–7	10, F	168	200	...	45	131	N
vii–12	19, F	138	151	45	29	70	L
vii–13	10, M	102	170	81	29	96	L
vii–14	19, M	105	151	41	35	88	N
vii–15	15, M	108	203	79	36	108	N
vii–16	13, M	107	196	76	32	105	L
vii–17	6, F	154	194	...	59	136	N
vii–18	25, F	174	228	...	61	150	N

* TC = total cholesterol. PL = phospholipids. TG = triglycerides.
† N = normal HDL. L = abnormally low. HDL (heterozygous). A = absent HDL (homozygous).

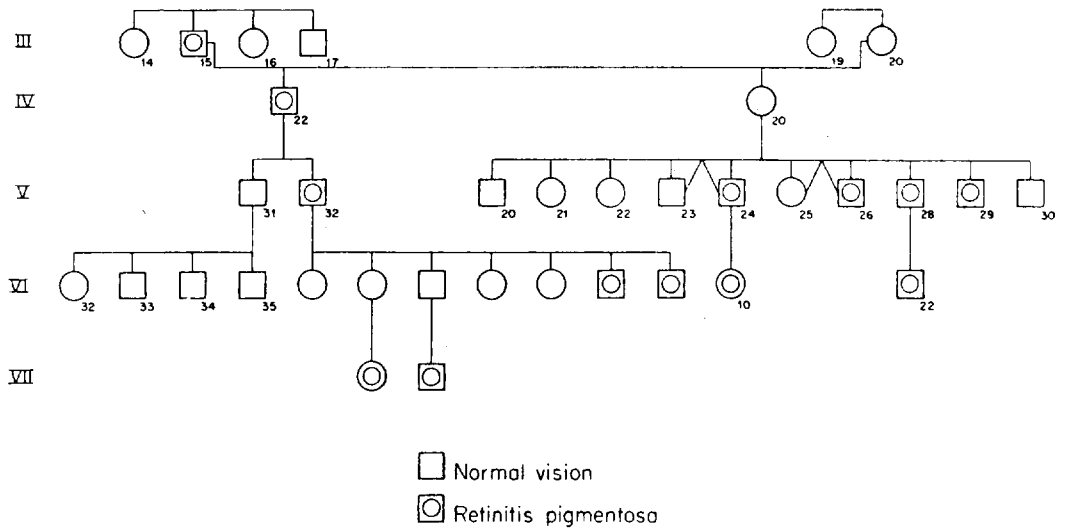

FIG. 5. Pedigree of Kentucky propositi, showing distribution of retinitis pigmentosa. Numbers are same as in Figure 4.

Virginia and the Carolinas. However, there were no names common to the population of Tangier Island nor were any traceable connections between the affected Kentucky, Missouri and Tangier kindreds discovered.

Blood samples were obtained in the nonfasting state for analysis of plasma HDL, as previously described [*4*]. The same criteria were employed for assignment of phenotype of HDL concentration as had been utilized in the study of the other two kindreds. HDL was quantified by the amount of cholesterol obtained in the preparative ultracentrifuge fraction of density greater than 1.063; and "abnormally low" values were defined as those below the lower 5 per cent of control values, or 33 and 35 mg. per 100 ml. in male and female subjects, respectively [*4*].

The distribution of HDL concentrations in the kindred is shown in Table VI and Figure 4. The mother of the propositi has a deficiency of HDL, as do relatives of both of the parents, who were second cousins. Judging by the number of his abnormal siblings, the father of the propositi was probably abnormal—a circumstance in keeping with the recessive mode postulated for Tangier disease from previous studies. In Figure 4 are two phenotypes inconsistent with the hypothesis that a single abnormal gene should cause deficiency of HDL. One (VII–16) is a heterozygote whose parents are normal. More importantly, the other (VII–14), the son of the patient in Case 1 (VI–27) who should be heterozygous abnormal, appears to be phenotypically normal. The HDL concentrations in both these subjects (Table VI) vary from the arbitrary limit of normal by only 2 mg. per 100 ml., which is within the 10 per cent experimental error of the methods [*4*].

The patient in Case 2 and his wife (VI–25) had only two children (VII–12, VII–13) both of whom have low plasma concentrations of HDL. There is no history of stillbirths or miscarriages to suggest less than normal viability of a homozygous abnormal offspring theoretically possible from this mating.

With allowance for the uncertainty in detecting the heterozygous phenotype, the data in the present pedigree indicate a familial abnormality in maintaining plasma HDL concentrations which is consistent with the genetic mode earlier postulated for Tangier disease [*4*].

RETINITIS PIGMENTOSA

A number of living family members have marked visual impairment. In every instance night blindness was first noted in adolescence or early adulthood; in most instances it progressed to near blindness. Several ophthalmologists who examined a number of the affected persons have described this disorder, commonly known among family members as "the moon eye," as retinitis pigmentosa.

The distribution of retinitis pigmentosa is shown in Figure 5. The retinopathy is associated with descendants of III–15. Seven of these descendants with retinitis were not available for blood sampling and are not included in the pedigree

chart. (Fig. 4.) Among those persons in whom the diagnosis of retinitis pigmentosa seems reasonably substantiated, the concentration of HDL is low in five and normal in one. It should be noted that both patients described herein were examined on several occasions by competent ophthalmologists, and no evidence of retinitis was seen. The data suggest that the defect in HDL and the retinitis pigmentosa are not linked in this kindred.

COMMENTS

There remains little doubt that the two adults described herein have the same genetically determined disorder described previously in children as Tangier disease. Each has a deficiency of plasma HDL quantitatively as marked as that in the children, persistent tags of abnormal-appearing tonsil tissue and foam cells in many tissues without evidence of accompanying granulomatous or other inflammatory changes. Excessive storage of cholesterol esters in the spleen of one patient has been demonstrated. The fatty acid moiety of these esters is also the same as in other cases, although it has not been proved that this is a useful basis for distinguishing the biochemical defect. Finally, many relatives have abnormally low plasma concentrations of HDL, the distribution being comparable to those found in the two pedigrees of the childhood cases [*4*].

The discovery of two more cases of Tangier disease is noteworthy for several reasons. It suggests that the condition may not be extremely rare and that physicians will find it worthwhile to keep in mind the simple clues to its diagnosis. These are hypocholesterolemia in the absence of malabsorption and in association with foam cell infiltration of tissues. A unique manifestation of the latter appears to be an orange or yellow-gray appearance of the tonsils or the remaining mucosa after these usually enlarged tissues have been removed. Thus the observation of unusual tonsils or the presence in the oropharynx of one or more follicles surrounded by a distinctive light orange or yellow-gray ring is an indication for determining the plasma cholesterol. This should also be carried out in any instance of unexplained enlargement of the liver, spleen or lymph nodes, of corneal infiltration or of abnormal appearing rectal mucosa.

If the cholesterol concentration is less than 125 mg. per 100 ml., HDL concentrations should be determined. Paper electrophoresis is a useful gross screening technic, but some of the usual staining technics define alpha$_1$ lipoprotein (the electrophoretic equivalent of HDL) less well than other lipoproteins and may falsely indicate absence of HDL when actually the concentration is just somewhat lower than normal. Starch-block electrophoresis is better for defining alpha$_1$ lipoprotein because larger amounts of plasma can be loaded, providing a deeper color with lipid stains.

Preparative ultracentrifugation provides accurate quantitation of HDL. However, the definition of a lipoprotein in the ultracentrifuge is an operational one, depending upon the appropriate combination of lipid and protein to impart a specific density to the molecules. There are instances, as in severe hyperglyceridemia [*19*] or obstructive liver disease [*20*], in which deficiency of HDL or even apparent absence may be secondary to a change in density or physical state of the lipoprotein rather than to an absolute decrease in quantity. Confirmatory diagnosis of severe HDL deficiency, therefore, depends upon immunochemical methods for identification of the lipoprotein. Pure antibodies to human HDL are not always readily available; but various anti-human serums are, and these may be used in immunoelectrophoresis with appropriate stains to identify the lipoproteins.

The present cases offer the first opportunity to gauge the long-term effects of a presumably congenital absence of significant circulating HDL. These adults have lived four times as long as the previously known patients. They demonstrate that while Tangier disease is a relatively benign process, compatible with reasonably normal health at least to the fifth decade, it can be associated with significant disability. The presenting clinical problem in Case 1 was splenomegaly. Initially the peripheral blood showed mild leukopenia with a normal differential, thrombocytopenia and increased red cell regeneration. These factors in conjunction with active marrow suggested mild hypersplenism. The hematologic findings became more abnormal and, together with progressive splenomegaly, led ultimately to splenectomy. Severe coronary heart disease was present in Case 2 without splenomegaly or hematologic abnormalities. Any influence of disturbed cholesterol transport and storage in the pathogenesis of this fatal disease is presently unknown. One child with Tangier disease (Case 3) has a congenital pulmonic stenosis [*3*].

Hitherto unappreciated sites of lipid storage in Tangier disease were demonstrated in the gut and eyes of the two adult patients. Gastrointestinal symptoms (mild intermittent diarrhea) in Case 1 led to proctoscopic examination, which revealed a unique gross appearance of the rectal mucosa in association with mucosal and submucosal foam cell infiltration. Identical findings were present in Case 2. The suggestively steatorrheal nature of the stools in Case 1 was investigated by an intake-excretion study of fecal fat and nitrogen. The value for fecal fat was slightly in excess of normal. (Table I.) Both of the brothers also had low serum concentrations of carotene. However, the absence of other evidence of malabsorption, despite roentgenography of the small intestine and biopsy of the jejunal mucosa in Case 1, militates against a primary or secondary malabsorption syndrome which might be responsible for the abnormal serum lipid and lipoprotein findings in these patients. Moreover, in Case 2 the patient had no gastrointestinal symptoms and was obese, and his fecal fat and nitrogen excretions were normal. No foam cells or other abnormalities were seen in the biopsy specimens of jejunal mucosa from either patient. Gut biopsies have not been performed in the four children with Tangier disease; but they, too, have had no evidence of gastrointestinal dysfunction.

The corneal clouding and stromal deposits observed on slit-lamp examination presumably are due to deposition of cholesterol esters in a manner similar to that occurring in other tissues. Moderate hyperuricemia without evidence of abnormal renal function was present in both cases, but its significance here cannot be determined.

The basic inheritable defect in Tangier disease remains to be established with certainty. HDL is one of the major mechanisms for transporting lipid in extracellular fluid, others being the albumin-free fatty acid complex and LDL [*21*]. From a physiologic standpoint, particulate emulsions such as chylomicrons may be considered a fourth mechanism, although their chemical and functional relationships to the other lipoproteins are not fully understood. One of the major contributions of studies of Tangier disease has been to point out an apparent lack of absolute requirement for HDL in the absorption of fat and formation of chylomicrons [*18*].

The polypeptide in HDL is specific and distinct from that in LDL. From the data available it seems most likely that inability to maintain significant concentration of plasma HDL in Tangier disease is due to a genetically determined defect in elaboration of this polypeptide. If this be true, the widespread accumulation of cholesterol esters in tissues suggests that HDL may have some specific function in maintaining the equilibrium of cholesterol between plasma and tissues.

It is noteworthy that there are also abnormalities in LDL in Tangier disease. In the adults they have been especially marked (Table v), but qualitatively similar findings have been present in the affected children [*18*]. The hypocholesterolemia in Tangier disease thus is not solely due to an absence of significant HDL, although the latter lipoprotein class is by far the most profoundly affected. In the ultimate explanation for Tangier disease as based on alteration at a single gene locus, it will be necessary to account for changes in all classes of lipoproteins as, at the least, secondary phenomena.

One of the few conditions in which cholesterol esters are stored selectively in reticuloendothelial tissues is eosinophilic granuloma [*22*]. Neither the diagnostic granulomatous changes nor other typical clinical features are found in Tangier disease. One of us (D. S. F.) has had opportunity to measure plasma HDL in two patients with an active form of the disseminated variety of eosinophilic granuloma. In one, HDL was normal; in the other, it was abnormally low (HDL cholesterol measured 24 mg. per 100 ml., the mean for her age and sex being 54). However, the decrease was not comparable to the near absence seen in Tangier disease [*23*]. Eosinophilic granuloma is not a familial disease, and tonsillar abnormalities have not been reported in association with it.

While the frequency of the gene or genes associated with Tangier disease must undoubtedly be quite low in the general population, the finding of six cases within a three year period of awareness of the disease suggests that more are to be found. It still is less common than its counterpart abnormality, abetalipoproteinemia [*24,25*]. The latter is characterized by the absence of both the LDL and particulate forms of lipoproteins and is associated with acanthocytosis, steatorrhea, neurologic changes and retinopathy. While LDL was reported as being deficient in the parents of the patient first described [*24*], no definite mode of genetic influence on the development of this latter disease has yet been established. The extension of clini-

cal observations in Tangier disease to adults suggests that severe deficiency of HDL is associated with a more benign long-term course than abetalipoproteinemia. Both of these diseases offer unusual opportunities for further exploring the functions and control of the major plasma lipoprotein systems, and all new patients deserve careful study and extensive documentation.

SUMMARY

Two brothers, aged forty-five and forty-eight, with familial deficiency of high-density lipoprotein (HDL) (Tangier disease) are described. These are the fifth and sixth known patients and the first adults with the disease. They demonstrated the hypocholesterolemia, abnormal tonsils and tissue storage of cholesterol esters characteristic of this condition. One brother had hypersplenism and both had unusual infiltration of the corneas and rectal mucosa, new findings presumably related to the chronic course of Tangier disease. One brother had coronary artery disease which apparently caused his death. Low plasma concentrations of high-density lipoprotein were distributed among family members in a manner similar to that reported in the two other involved kindreds.

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